Departement für Nutztiere, Klinik für Reproduktionsmedizin

der Vetsuisse-Fakultät Universität Zürich

Vorsteher: Prof. Dr. med. vet. Heiner Bollwein
Direktor: Prof. Dr. med. vet. Heiner Bollwein

Arbeit unter wissenschaftlicher Betreuung von Dr. med. vet. Maren Feldmann

Pathogen-specific changes of milk composition and lack of effect of high dosage oxytocin during mastitis in dairy cows

Inaugural-Dissertation

zur Erlangung der Doktorwürde der

Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Florian Johannes Strasser

Tierarzt

aus Deggendorf, Deutschland

genehmigt auf Antrag von

Prof. Dr. med. vet. Heiner Bollwein, Referent

PD Dr. med. vet. habil. Maike Heppelmann, Korreferentin

2019

Meiner Familie.

INDEX

Abstract

Vetsuisse Faculty University of Zurich (2019)

Florian Johannes Strasser

Department for Farm Animals, Clinic of Reproductive Medicine

cgeisinger@vetclinics.uzh.ch

Pathogen-specific changes of milk composition and lack of effect of high dosage oxytocin during mastitis in dairy cows

The objective of the present study was to investigate changes of the blood derived milk components somatic cell count (SCC), lactate dehydrogenase (LDH) and especially immunoglobulin G (IgG) in milk of cows suffering from mastitis caused by different pathogens. In addition, we have investigated the effects of repeated i.v. injections of a high dosage of oxytocin (OT; 100 IU) on these parameters. Therefore, milk samples from all udder quarters of 184 dairy cows from several dairy farms were collected at day 1, which was the day of the clinical examination and diagnosis of mastitis and days 2, 3, 14 and 28. Bacteriological examination on day 1 detected involved pathogens. Cows were randomly assigned into treatment group (OT injections on days 1 and 2) and control group (no OT). Changes of SCC, LDH and IgG were shown to be pathogen dependent. Highest values of all three parameters were measured in mastitis caused by *Streptococcus uberis*. The changes were less pronounced in mastitis cases with other *Streptococci spp.*, *Staphylococci spp.* or *Corynebacterium bovis.* Oxytocin treatment did not affect any parameter from days 1 to 3 independent of the pathogen. Only in quarters infected with other *Staphylococci* than *Staphylococcus aureus* a decreased SCC was observed between days 2 and 14 as well as an increase of IgG in quarters, where no pathogens were detected. Thus, a high dosage of OT as a treatment for mastitis is obviously not sufficient.

Key words: dairy cow, mastitis, oxytocin, pathogen specifity

Zusammenfassung

Vetsuisse-Fakultät Universität Zürich (2019)

Florian Johannes Strasser

Departement für Nutztiere, Klinik für Reproduktionsmedizin

cgeisinger@vetclinics.uzh.ch

Pathogen-spezifische Veränderungen in der Milch und Einsatz von hochdosiertem Oxytocin zur Therapie von Mastitiden bei Milchkühen

Ziel der Studie war die Erfassung pathogenabhängiger Veränderungen der somatischen Zellzahl (ZZ), der Laktatdehydrogenase (LDH) und von Immunglobulin G in der Milch von Kühen, die an Mastitis erkrankt waren. Ausserdem wurde der Effekt einer mehrfachen i.v. Injektion von hochdosiertem Oxytocin (OT; 100 IU) auf diese Parameter analysiert. Dazu wurden 184 Kühe aus verschiedenen Milchviehbetrieben entweder der Behandlungsgruppe mit OT Injektion an den Tagen 1 und 2 oder der Kontrollgruppe ohne OT Behandlung zugeordnet. Milchproben aller Euterviertel wurden an Tag 1, an dem auch die klinische Untersuchung und Diagnose erfolgte, sowie an den Tagen 2, 3, 14 und 28 genommen. Mit Hilfe einer bakteriologischen Untersuchung an Tag 1 wurden die Pathogene ermittelt. Die Veränderungen von ZZ, LDH und IgG waren pathogenabhängig. Die jeweils höchsten Werte wurden bei *Streptococcus uberis* gemessen. Sie waren weniger stark verändert bei anderen *Streptococci spp*., *Staphylococci spp*. oder *Corynebacterium bovis*. Unabhängig vom verursachenden Pathogen bewirkten die OT Behandlungen keine Veränderungen der untersuchten Parameter an den Tagen 1 bis 3. Lediglich bei Infektionen mit anderen *Staphylococci* als *Staphylococcus aureus* konnte ein Rückgang der ZZ zwischen Tag 2 und Tag 14, sowie ein Anstieg von IgG in der Milch der nicht infizierten Viertel beobachtet werden. Eine Therapie mit hochdosiertem OT bei Mastitiden ist deshalb als nicht ausreichend zu bewerten.

Stichwörter: Milchkuh, Mastitis, Oxytocin, Pathogenspezifität

Pathogen-specific changes of milk composition and lack of effect of high dosage oxytocin during mastitis in dairy cows

Authors
F.J. Strasser, M. Feldmann, J.J. Gross, A.T.M. Müller, H. Pfingstner, S. Corti, R. Stephan, H. Bollwein, R.M. Bruckmaier, O. Wellnitz

INTRODUCTION

In dairy cows, many different pathogens can cause clinical and subclinical mastitis. During an infection of the mammary gland, milk composition usually changes. To achieve high standards of milk quality in dairy farming, mastitis is commonly treated with antibiotics. As the use of antibiotic treatment in food producing animals is progressively losing acceptance due to the possible selection of resistant pathogens as well as the possible occurrence of residues, the goal is to reduce antibiotic usage in veterinary practice. Furthermore, the animal´s own immune system should be promoted to cope with invading pathogens. However, non-antibiotic mastitis treatment is usually adjusted to the severity of mastitis rather than to the pathogens involved, e.g. anti-inflammatory medication or pain management. With increasing knowledge on pathogen specific effects of non-antibiotic treatments, their efficiency and appropriate use could be optimized.

Oxytocin (**OT**) is a hormone, which is released from the pituitary gland in response to teat stimulation by the calf or the milking machine (Crowley and Armstrong, 1992; Bruckmaier and Blum, 1998). It causes contraction of myoepithelial cells, which surround the alveoli formed by the milk secreting epithelial cells. This contraction leads to milk ejection (Soloff et al., 1980). In veterinary practice OT administration at a supraphysiological dosage is used to induce a maximum possible milk ejection. The positive effect on mastitis cure was suggested to be due to the promotion of complete udder emptying, which leads also to a removal of the bacteria (Knight et al., 2000). Exogenous OT administered at an extremely high supraphysiological level up to 100 IU was previously shown to increase the permeability of the blood-milk barrier (Allen et al., 1990; Wall et al., 2016). The mechanism behind this

phenomenon is supposed to be a partial breakdown of the tight junctions between neighboring mammary epithelial cells (Linzell and Peaker, 1971). Furthermore, OT has recently been reported to induce mammary epithelial disruption and epithelial cell exfoliation (Herve et al., 2018).

The increased permeability of the blood-milk barrier is characterized by the appearance of blood constituents in milk because of the facilitated paracellular diffusion of blood and milk constituents (Nguyen and Neville, 1998). The blood constituents that are transferred from blood into milk include the protein serum albumin (**SA**) and the enzyme lactate dehydrogenase (**LDH**; Stelwagen et al., 1994; Lehmann et al., 2013). Their increasing appearance in milk has, therefore, the potential to be used as a marker for an increased permeability of the blood-milk barrier. In addition, immunoglobulins (**Ig**) are increasingly transferred from blood into milk through an impaired blood-milk barrier (Lehmann et al., 2013). In milk these Ig, specifically IgG, are supposed to have functional properties during a mammary immune response (Burton and Erskine, 2003). The increase of IgG-transfer into milk could, therefore, enhance the immune competence of the mammary gland if antibodies against mastitis pathogens are present in the blood, i.e. in vaccinated cows and, could improve the cure rates of mastitis.

A recent study showed that the administration of OT in supraphysiological doses causes an increase of the somatic cell count (**SCC**) in milk of healthy cows and also in cows with induced mastitis by endotoxins from Gram-negative and Gram-positive bacteria (Wall et al., 2016). These are mainly cells of the innate immune system (Sarikaya et al., 2004) which have significant effects in combatting against udder pathogens (Burton and Erskine, 2003). Therefore, a pronounced increase of SCC during mastitis by OT application could improve the elimination of pathogens.

The effects of high dosages of OT on SCC and the blood-milk barrier permeability were shown to be pathogen specific (Wall et al., 2016). Therefore, it is likely that effects of mastitis treatment with OT is pathogen dependent. Until now pathogen dependent changes of milk composition including the antibody content in milk during mastitis after OT administration were mainly investigated in experimental models with pathogen associated molecular patterns (PAMP; Wellnitz et al., 2011; 2013). Nevertheless, little is known on effects of OT on changes of the blood-milk barrier and SCC during mastitis with different live pathogens. Therefore, the aim of the present study was to investigate changes in concentrations of blood-derived components like SCC,

LDH, and IgG in milk of cows with naturally occurring mastitis caused by different pathogens, treated with or without intravenous administration of high dosages of OT.

MATERIALS AND METHODS

Animals

In 2017, lactating Simmental cows (n=184) with signs of mastitis like clots and flakes in milk, painful and swollen udder quarters with or without disruption of general condition or only an increased SCC without further symptoms were presented to two veterinarian practices in Zachenberg, Germany, and Oberkappel, Austria. The domains of the practices were located in a distance of less than 100 km from each other. Climatic conditions and feed ingredients for dairy cows were comparable among farms. The cows included in the present study were housed on 59 farms with a size of four to 200 dairy cows, equally distributed in different housing forms: all year tie stall housing, all year free stall housing, free stall and pasture, tie stall and pasture, or a combination of tie and free stall. Cows were included in the study if they were less than 250 DIM, and had no mastitis treatment in the last four weeks. Cows were not vaccinated against mastitis pathogens. The study was approved by the District Government of Upper Bavaria, Germany (registration number: AZ 55.2-1-54-2532-4-2017) and the Office of the Upper Austrian State Government, Austria (registration number: Ges-2016-409034/5-Ho).

Experimental Design

After diagnosis and a careful clinical examination on day 1 general condition, body temperature, changes in udder condition or milk quality, and other significant findings were recorded and cows were further examined on days 2, 3, 14 and 28.

Cows were randomly assigned to the treatment group (OT; n = 101) or to the control group (n = 83).

Milk samples from all four quarters were collected on days 1 (prior to the treatment), 2, 3, 14, and 28. Immediately after sampling, cows of the treatment group (OT) received 100 IU of oxytocin intravenously on days 1 and 2. These cows were not milked before, or immediately after the injections. If the mastitis was acute, i.e. cows showed clots or flakes in the milk and the general condition of animals was

affected, cows were treated independently of the assigned experimental group according to the common therapy protocol of the veterinary practice (including antibiotic treatment), directly after collecting the samples on day 1. If possible, i.e. if the general condition was not impaired, cows were not treated, until the result of the bacteriological examination of the milk sample was available. The cows were not milked after the oxytocin injection before the next scheduled milking.

Laboratory Analyses

On days 1, 2, 14, and 28 the SCC was measured in the milk samples with a DeLaval cell counter (DCC; DeLaval, Tumba, Sweden) immediately after sampling. Measurements above 3×10^6 cells/mL were recorded as $>3 \times 10^6$ cells/mL as the detection limit for the counting device is between $3 - 4 \times 10^6$ cells/mL. Results were log-transformed ($\log_{10}$) to assure normal distribution and to take into account that higher cell counts above the detection limit could not be captured. On day 3 SCC was solely estimated with a California Mastitis Test (**CMT**; Schalm and Noorlander, 1957). After SCC measurement milk samples were stored for further analyses at -20°C.

For the measurement of LDH activity milk serum was obtained by centrifugation of the milk samples at 1,900 x g for 15 min at 4 °C and then at 20,800 x g for 30 min at 4 °C. The LDH activity was determined using the commercial kit AXON00025 (Axon-Lab AG, Baden, Switzerland) using an automated analyzer (Cobas Mira, Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol.

Milk samples from 299 quarters of 97 cows were selected for the measurement of IgG concentrations. These cows were either treated with OT (n= 57) or used as controls with no OT treatment (n=40). The IgG concentrations were measured by ELISA kit No. E10-118 (Bethyl Laboratories, Montgomery, TX) according to the manufacturer`s instructions. For the absorbance measurement a Synergy Mx plate reader (BioTek Instruments, Winooski, VT) was used. The inter- and intra-assay coefficients of variation were 4.8% and 8.9%, respectively.

Bacteriological examinations of the milk samples from days 1 and 28 were performed according to standard procedures (Anonymous, 1999).

Statistical Analysis

Quarters were grouped by pathogens based on their quarter milk bacteriological results on day 1. Results are presented as lsmeans ± SEM. Statistical analyses were performed by using SAS (SAS version 9.4 SAS Institute Inc., Cary, NC).

The general linear models (GLM) procedure was used to test the effects of treatment, day, and pathogen. Correlation between LDH and SCC, and IgG and LDH were evaluated by the CORR procedure. Differences of means of IgG concentrations, SCC, and LDH activity in milk between treatment days and bacteriological negative and infected with Gram-positive bacteria were tested for significance using paired t-test procedure. For SCC and LDH quarters were grouped by treatment (with or without OT treatment) and by pathogens. To test differences in IgG concentrations between days quarters infected with Gram-positive bacteria in which IgG was measured were merged to one category to increase the number of observations as IgG was not measured in all samples. Differences were considered significant when $P < 0.05$.

RESULTS

Pathogens and SCC

Detected pathogens and the SCC in affected quarters on day 1 are shown in table 1. Results were obtained from 700 quarter samples. In 539 quarters, there was no pathogen detectable. In 61% of these quarters the SCC was above 100,000/mL and in 14% of these quarters the SCC above the detection limit of the DCC system (≥3,000,000/mL). Pathogens were found in 161 quarters, thereof 82 quarters were treated with oxytocin.

The SCC on days 1, 2, 14, and 28 in quarters infected with different pathogens with and without OT treatments are shown in table 2. As SCC values that were at or above detection limit were calculated as 3,000,000 cells/mL the results were logarithmized (log10) and the number of samples with SCC at or above 3,000,000 cells/mL in each group is also shown on table 2. The OT treatment, the day, and the pathogen influenced the SCC ($P < 0.05$, <0.001, <0.001, respectively). However, only in quarters infected with other *Staphylococcus* than *Staphylococcus aureus* a significant decrease ($P < 0.05$) of the SCC between days 2 and 14 was detectable with OT

treatment. In control quarters that were not treated with OT there was no significant change (P=0.54) of SCC between day 2 and day 14 detectable. The SCC means of groups treated with OT compared to groups without OT treatment on day 1 within pathogen was not different except in *Streptococcus uberis* infection. The CMT results on day 3 were between ++ and +++ in quarters infected with the different pathogens. The results of CMT within pathogens were not different between treatment with or without OT.

LDH activity in milk

Mean LDH activity measurements in milk from quarters infected with different pathogens within treatment group on the different days are shown in table 3. LDH activity in milk was positively correlated (P<0.05) with SCC in infections with all different pathogens except in quarters infected with *Staphylococcus aureus*. Highest LDH activities were found in milk samples of glands infected with Gram-negative bacteria and with *Streptococcus dysgalactiae* and *Streptococcus uberis*. The OT treatment had no effect on LDH concentrations, whereas the day and the pathogen had significant effects on LDH concentrations in milk (P < 0.001).

The Pearson correlation coefficients between LDH activity and IgG concentrations over all samples on the first three days in milk were 0.54 (P=0.007), 0.61 (P<0.001), 0.97 (P<0.001), 0.89 (P<0.001), and 0.01 (P=0.295) in quarters infected with *Corynebacterium bovis*, *Staphylococcus aureus*, other *Staphylococcus*, *Streptococcus uberis* and in quarters where no pathogens were detected, respectively.

IgG concentrations

Mean IgG concentrations in milk of quarters infected with different pathogens within treatment group on the different days are shown in figure 1. If cows were treated with OT the IgG concentration in the 128 quarters with negative bacteriological results changed (P < 0.05) from concentrations between 0.13mg/mL and 3.14mg/mL on day 1 to concentrations between 0.31mg/mL and 2.65mg/mL on day 2. There was no further change of IgG concentrations on day three. In quarters of OT treated cows that were diagnosed with Gram-positive bacteria the IgG concentrations ranged from 0.16 mg/mL and 11.24 mg/mL on day 1 and did not significantly change on day 2 or day 3.

Highest IgG concentrations were measured in a quarter that was infected with *Staphylococcus spp*. (11.24 mg/ml and 11.68 mg/mL on day 1 and day 2, respectively). Very high IgG concentrations were measured in quarters infected with *Streptococcus uberis* (up to 10.99 mg/mL). In these measurements three quarters of one cow that was in the 9th lactation were included. IgG concentrations were not measured in quarters infected with *Escherichia coli.*

OT treatment did not have an effect on IgG concentrations on days 2 and 3 within groups of involved pathogens (see figure 1).

Other symptoms

All cows with *Escherichia coli* infection had increased rectal temperature above 39.5 °C. Furthermore, 22.7% of cows infected with *Streptococcus uberis*, and 17.6 % of cows infected with *Streptococcus dysgalactiae* developed this temperature increase. In all other infections less than 12% of cows developed a rectal temperature above 39.5 °C.

Clots and flakes were found in milk from all *Escherichia coli* infected quarters. Fewer quarters with flakes were found in *Streptococcus uberis* (57.7%), *Streptococcus dysgalactiae* (52.9%) and other *Staphylococcus* (44.4 %), and lowest numbers of quarters with flakes were found in *Staphylococcus aureus* infections (31.4%).

In the OT treatment group 9, 10, 3, 6, and 3 quarters and in the control group 4, 3, 5, 5, and 11 quarters infected with *Corynebacterium bovis, Staphylococcus aureus, Streptococcus dysgalactiae*, other *Staphylococcus spp.*, *Streptococcus uberis,* respectively, were treated with antibiotics.

DISCUSSION

Different mastitis pathogens were detected in milk from dairy cows in the present study. The main bacteria that induced mastitis were *Streptococcus uberis*, *Staphylococcus aureus*, and other *Staphylococci*. This represents the expected distribution of mastitis pathogens in dairy farming throughout Europe (Heikkilä et al., 2018). It also shows that *Streptococcus uberis* is increasingly a significant inducer of mastitis

compared to older studies (Tenhagen et al., 2006). Interestingly, 61% of the quarters in which no pathogens were detected had an SCC above 100,000 cells/mL, and 14% of these bacteriological negative quarters had an SCC above the detection limit of the used device, which is at 3,000,000 cells/mL. According to the German Veterinary Medical Society (DVG, 2009) an SCC below 100,000 cells/mL in quarter foremilk samples is in the physiological range and with increasing SCC above 100,000 cells/mL the likelihood of an existing mastitis increases. As only standard methods for bacterial detection were used in this study, it is possible that quarters were infected with pathogens that are not captured with these methods, e.g. *Mycoplasma*. Furthermore, Hill (1981) showed that in severe mastitis induced by *Escherichia coli* the bacteria are often eliminated from the mammary gland that only endotoxin and no live bacteria can be found in mastitic milk. Therefore, in the quarters from which sterile milk samples were received, Gram-negative bacteria might have had induced an inflammation but they were already eliminated by the immune system of the mammary gland. Other reasons for not detecting bacteria in milk from infected mammary glands could be the varying shedding of bacteria into the milk as it is seen for example in *Staphylococcus aureus* infections (Walker et al., 2011).

Independently from the involved pathogen SCC, LDH, and IgG was not influenced by OT treatment from the first to the second day of investigation. Except a decreased SCC in quarters infected with other Staphylococci than *Staphylococcus aureus* was seen after two weeks with OT treatment. It was shown before that the SCC increased in healthy quarters after injection of 100 IU OT (Wall et al., 2016). In addition, in quarters which were immunologically stimulated by intramammary application of the endotoxin lipopolysaccharide from *Escherichia coli* or with lipoteichoic acid from *Staphylococcus aureus* the high dosage of OT further increased the SCC in addition to the increase in response to the endotoxin challenge. However, in these experimentally induced immune responses, the mammary glands were healthy before the start of experiment. It is likely that a pronounced increase of SCC could not be detected in the present study because the cows in this study were presented to the veterinarians and first sampling occurred when first symptoms were detected by the farmer. At that time the increase of SCC was already triggered and in process. When exactly the invasion of the pathogens occurred is not known, and the first sampling was, therefore, performed at different stages of mastitis. Another reason for

not detecting a pronounced SCC increase after OT injection in spontaneous mastitis compared to experimental induced mastitis could be that the concentration of intramammarily applied endotoxins within the mammary gland continuously decreases beginning immediately after infusion with ongoing milk synthesis. In contrast, live bacteria are continuously multiplying which represents an ongoing and increasing immune challenge. This progeny of bacteria is pathogen specific (Bannerman et al., 2004) and is accompanied with a pathogen specific pattern of SCC increase. At which stage of mastitis the sampling and the OT injection was performed cannot be determined.

The intravenous application of 100 IU oxytocin to dairy cows was expected to pronounce the opening of the blood milk barrier, and to increase, therefore, not only the SCC, but also the IgG content in milk (Wall et al., 2016). Antibody binding to bacteria opsonizes pathogens to facilitate the phagocytosis by leukocytes (Targowski, 1983). Therefore, the increase of IgG in milk during mastitis could support the combat against the involved pathogens if specific antibodies would be available in the blood (Burton and Erskine, 2003), e.g. after previous contact with the pathogen or after vaccination. In this field study, the treatment of dairy cow mastitis with 100 IU oxytocin intravenously did not show significant effects on the increase of IgG after the treatment. Only in healthy quarters, an increase of IgG by OT treatment was detected. Concentrations of IgG and their increase in milk during an ongoing mastitis are very individual (Wall et al., 2016). This was also seen in varying concentrations on day 1 in this study. Furthermore, as already discussed for SCC, the first sampling was performed at different stages of mastitis, which can mask a further increase of IgG transfer from the blood.

It is known that different bacteria induce the mammary immune response differently, which leads to a different expression of immune factors in the mammary gland and to different concentrations of several immune factors and blood components in milk (Bannerman et al., 2004; Wellnitz and Bruckmaier, 2012). In the present study highest values of SCC, LDH, and IgG were measured in *Streptococcus uberis* infected quarters. Very high concentrations of IgG in milk of *Streptococcus uberis* infected quarters of one cow in the 9th lactation were found. It is known that IgG1 concentrations in serum increase after the third lactation (Caffin et al., 1983); therefore, the age of this cow could be one of the reasons for the high IgG concentrations. It is also known that IgG decreases throughout lactation (Musayeva et al., 2016) and

this cow was only 40 days in milk. Furthermore, it was shown that cows with mastitis induced by *Streptococcus uberis* have higher IgG concentrations in serum than cows with mastitis induced by *Staphylococcus aureus* (Kociņa et al., 2012). In addition, it is not known if this cow had other infections besides the mastitis that could be an additional reason for the high IgG concentrations in milk.

The enzyme LDH in milk is known to increase during a mammary immune response and it originates at least partially from soluble LDH in the blood through an opening of the blood milk barrier (Lehmann et al., 2013). However, LDH is also released by damaged cells (Glick, 1969; Lehmann et al., 2013). Thus, the origin of LDH in milk shows the opening of the blood-milk barrier on the one hand and the increased number of leukocytes in milk (Kato et al., 1989) and cell damage in the udder (Bogin et al., 1977; Zank & Schlatterer, 1998) on the other hand. This leads to correlations between the LDH activity in milk and SCC during mastitis, which was also shown in this field study. As the opening of the blood-milk barrier and the increase of SCC during mastitis is dependent on the involved pathogen (Barkema et al., 1998; Wellnitz et al., 2011, 2016) this also explains why this correlation was found in infections with most of the pathogens but not in *Staphylococcus aureus* induced mastitis. Furthermore, the correlation between LDH activity and IgG concentration in milk were different in quarters infected with different pathogens. The correlations were similar with results found by Hernàndez-Castellano et al. (2017) in *Corynebacterium bovis*, *Staphylococcus aureus*, and *Streptococcus uberis* infected quarters. In quarters infected with Gram-negative bacteria, highest SCC were expected (Barkema et al., 1998). The tremendous increase of SCC can lead to the higher LDH activity in milk from quarters infected with Gram-negative compared to those with Gram-positive bacteria. Furthermore, Gram-negative bacteria are assumed to impair the blood-milk barrier to a greater extent than Gram-positive bacteria (Wellnitz et al., 2013) which leads to an increased transfer of LDH from blood into milk. This also explains the high LDH concentrations in milk from Gram-negative infected quarters. Interestingly, also in quarters infected with *Streptococcus uberis* und *Streptococcus dysgalactiae* high LDH concentrations were found which is likely associated with the strong increase of SCC but can also indicate a relevant disruption of the blood milk barrier and/or tissue damage.

As LDH activity in milk has been considered as an indicator of udder health (Chagunda et al., 2006) it could be used in automatic milking systems to monitor

mastitis. The results show, however, that LDH is not a good marker for all mastitis pathogens to detect infections but clearly indicates severe mastitis with high SCC and tissue damage. Furthermore, the measurement of LDH activity to predict high IgG concentrations in milk, which could be important for the course of the disease, is not useful for all common mastitis pathogens.

Clots and flakes in milk were mainly found in quarters that were infected with *Streptococcus uberis* and *Escherichia coli*. These bacteria are known to induce severe mastitis. Therefore, the treatment with high dosages of OT does not seem to be a sufficient therapy to treat mastitis; however, supportive effects on cure rates are possible and should be further investigated.

CONCLUSIONS

The present study proves that changes of blood components like SCC, LDH, and IgG in milk during mastitis of dairy cows are pathogen dependent. The treatment of spontaneously occurring mastitis with high dosages of oxytocin does not sufficiently increase the IgG concentrations in milk to assume a positive effect on mastitis cure rates if antibodies against the involved pathogen are available. Solely in healthy quarters an increase of IgG was induced by OT but not in infected glands when treatment was performed during an already ongoing infection. Furthermore, an increase of SCC in response to injection of high dosages of OT, which could have a positive effect on the elimination of specific bacteria, could not be seen in this study. Obviously, the effects of OT in supraphysiological dosages on mastitis depend on the stage of the disease at which the injections are performed.

The results of this field study show that the treatment of mastitis with high dosages of OT as a stand-alone therapy in the field is obviously not sufficient. However, positive effects of high doses of oxytocin on mastitis cure rates cannot be excluded and should be further investigated.

REFERENCES

Allen, J. C. 1990. Milk synthesis and secretion rates in cows with milk composition changed by oxytocin. J. Dairy Sci. 73:975-984

Anonymous: Laboratory handbook on bovine mastitis. 1999, National Mastitis Council, Verona, WI

Bannerman, D. D., M. J. Paape, J. W. Lee, X. Zhao, J. C. Hope, and P. Rainard. 2004. *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. Clin. Diagn. Lab. Immunol. 11:463–472 doi:10 .1128/ CDLI .11 .3 .463 -472 .2004.

Barkema, H. W., Y. H. Schukken, T. J. G. M. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk tank milk somatic cell counts. J. Dairy Sci. 81: 411-419

Bogin, E., G. Ziv, J. Avidar, B. Rivertz, S. Gordin, and A. Saran. 1977. Distribution of lactate dehydrogenase isoenzymes in normal and inflamed bovine udders and milk. Res. Vet. Sci. 22:198-200

Bruckmaier, R. M., and J. W. Blum. 1998. Oxytocin release and milk removal in ruminants. J. Dairy Sci. 81:939–949

Burton, J. L., and R. J. Erskine. 2003. Immunity and mastitis. Some new ideas for an old disease. Vet. Clin. North Am. Food Anim. Pract. 19:1-45

Caffin, J. P., B. Poutrel, and P. Rainard. 1983. Physiological and pathological factors influencing bovine immunoglobulin G1 concentration in milk. J. Dairy Sci. 66:2161–2166 doi:10.3168/jds.S0022-0302(83)82063-3

Chagunda M. G., T. Larsen, M. Bjerring, and K. L. Ingvartsen. 2006. L-lactate dehydrogenase and N-acetyl-beta-D-glucosaminidase activities in bovine milk as indicators of non-specific mastitis. J. Dairy Res. 73:431-440

Crowley, W. R., and W. E. Armstrong. 1992. Neurochemical regulation of oxytocin secretion in lactation. Endocr. Rev. 13:33-65

DVG (Deutsche Veterinärmedizinische Gesellschaft) (2009): Isolierung und Identifizierung von Mastitiserregern. Leitlinien zur Entnahme von Milchproben unter antiseptischen Bedingungen und Isolierung und Identifizierung von Mastitiserregern. Verlag der Deutschen Veterinärmedizinischen Gesellschaft e.V., Gießen, 21-91.

Glick, J. H. Jr. 1969. Serum lactate dehydrogenase isoenzyme and total lactate dehydrogenase values in health and disease, and clinical evaluation of these tests by means of discriminant analysis. Am. J. Clin. Pathol. 52:320-328.

Heikkilä A. M., Liski E, Pyörälä S, Taponen S. 2018. Pathogen-specific production losses in bovine mastitis. J Dairy Sci. Aug 16. pii: S0022-0302(18)30752-5. doi: 10.3168/jds.2018-14824.

Hernàndez-Castellano, L. E., S. K. Wall, R. Stephan, S. Corti, and R. M. Bruckmaier. 2017. Milk somatic cell count, lactate dehydrogenase activity, and immunoglobulin G concentration associated with mastitis caused by different pathogens: A field study. Schweiz. Arch. Tierheilkd. 159:283–290.

Herve, L., V. Lollivier, H. Quesnel, and M. Boutinaud. 2018. Oxytocin induces mammary epithelium disruption and could stimulate epithelial cell exfoliation. J. Mammary Gland Biol. Neoplasia. 23:139-147. doi: 10.1007/s10911-018-9400-8.

Hill, A. W. 1981. Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. Res. Vet. Sci. 31:107-112

Kato, K., K. Mori, and N. Katoh. 1989. Contribution of leukocytes to the origin of lactate dehydrogenase isozymes in milk of bovine mastitis. J. Vet. Sci. - Japan 51:530-539.

Knight, C. H., J. L. Fitzpatrick, D. N. Logue, and D. J. Platt. 2000. Efficacy of two non-antibiotic therapies, oxytocin and topical liniment, against bovine staphylococcal mastitis. Vet. Rec. 146:311-316

Kociņa, I., V. Antāne, and I. Lūsis. 2012. The Concentration of immunoglobulins A, G, and M in cow milk and blood in relation with cow seasonal keeping and pathogens presence in the udder. Proc. Latv. Univ. Agr. 27:44-53 doi: 10.2478/v10236–012–0006–1

Lehmann M., O. Wellnitz, and R. M. Bruckmaier. 2013. Concomitant lipopolysaccharide-induced transfer of blood-derived components including immunoglobulins into milk. J. Dairy Sci. 96:889-896

Linzell, J. L., and M. Peaker. 1971. The effects of oxytocin and milk removal on milk secretion in the goat. J. Physiol. 216:717-734

Musayeva, K., A. Sederevičius, R. Želvytė, I. Monkevičienė, D. Beliavska-Aleksiejūnė, and S. Kerzienė. 2016. Concentration of lactoferrin and immunoglobulin G in cows' milk in relation to health status of the udder, lactation and season. Pol. J. Vet. Sci. 19:737-744 doi.org/10.1515/pjvs-2016-0093

Nguyen, D. D., and M. C. Neville. 1998. Tight junction regulation in the mammary gland. J. Mammary Gland Biol. Neoplasia 3:233-246

Sarikaya, H., C. Prgomet, M. W. Pfaffl, and R. M. Bruckmaier. 2004. Differentiation of leukocytes in bovine milk. Milchwissenschaft 59:586–589

Schalm, O., and D. Noorlander. 1957. Experiments and observations leading to the development of California mastitis test. J. Am. Vet. Med. Assoc. 130:199-204

Soloff, M. S., J. Chakraborty, P. Sadhukan, D. Senitzer, M. Wieder, M. J. Fernstrom, and P. Sweet. 1980. Purification and characterization of mammary myoepithelial and secretory cells from the lactation rat. Endocrinology 106:887–899

Stelwagen, K., I. Politis, J. H. White, B. Zavizion, C. G. Prosser, S. R. Davis, and V. C. Farr. 1994. Effect of milking frequency and somatotropin on the activity of plasminogen activator, plasminogen, and plasmin in bovine milk. J. Dairy Sci. 77:3577-3583

Targowski, S. P. 1983. Role of immune factors in protection of mammary gland. J. Dairy Sci. 66:1781-1789

Tenhagen, B.-A., G. Köster, J. Wallmann, and W. Heuwieser. 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci. 89:2542-2551

Walker, J. B., P. J. Rajala-Schultz, W. L. Walker, J. L. Mathews, W. A. Gebreyes, and F. J. DeGraves. 2011. Variation in daily shedding patterns of Staphylococcus aureus in naturally occurring intramammary infections. J. Vet. Diagn. Invest. 23:1114-1122

Wall, S. K., O. Wellnitz, L. E. Hernández-Castellano, A. Amphadpour, and R. M. Bruckmaier. 2016. Supraphysiological oxytocin increases the transfer of immunoglobulins and other blood components to milk during lipopolysaccharide- and lipoteichoic acid-induced mastitis in dairy cows. J. Dairy Sci. 99:9165-9173

Wellnitz, O., E. T. Arnold, and R. M. Bruckmaier. 2011. Lipopolysaccharide and lipoteichoic acid induce different immune responses in the bovine mammary gland. J. Dairy Sci. 94:5405-5412

Wellnitz, O., E. T. Arnold, M. Lehmann, and R. M. Bruckmaier. 2013. Short communication: Differential immunoglobulin transfer during mastitis challenge by pathogen-specific components. J. Dairy Sci. 96:1681-1684

Wellnitz, O., and R. M. Bruckmaier. 2012. The innate immune response of the bovine mammary gland to bacterial infection. Vet. J. 192: 148-152

Wellnitz, O., C. Zbinden, X. Huang, and R. M. Bruckmaier. 2016. Short communication: Differential loss of bovine mammary epithelial barrier integrity in response to lipopolysaccharide and lipoteichoic acid. J. Dairy Sci. 99:4851-4856

Zank, W., and B. Schlatterer. 1998. Assessment of subacute mammary inflammation by soluble biomarkers in comparison to somatic cell counts in quarter milk samples from dairy cows. J. Vet. Med. A 45:41-45

Table 1: Type (Pathogen), number (n), percentage of bacteria and mean somatic cell count (SCC) of infected udder quarters and number of animals treated with oxytocin (OT); bacteria were detected in 161 out of 700 examined quarters of 184 cows on day 1 (=day of diagnosis of clinical mastitis).

Pathogen	n	%	OT (n)	SCC (cells x 1000/mL)
Corynebacterium bovis	14	2.0	10	1,210 ± 308
Escherichia coli	7	1.0	2	>3,000
Staphylococcus aureus	35	5.0	18	1,389 ± 185
other *Staphylococcus*	39	5.6	25	691 ± 123
Streptococcus dysgalactiae	17	2.4	6	1,957 ± 289
Streptococcus uberis	44	6.3	12	1,676 ± 186
Klebsiella	1	0.1	0	2,400
Yeast	1	0.1	1	>3,000
Streptococcus agalactiae	1	0.1	0	>3,000
Trueperella pyogenes	1	0.1	0	>3,000
Gram-positive rods	1	0.1	0	253

Table 2: Somatic cell count (cells x 1000/mL) of udder quarters (n) in different pathogen and treatment groups (with oxytocin; without oxytocin) on different days relative to clinical diagnosis of mastitis (= day 1); *: number of quarters with SCC > 3'000'000/mL.

	with oxytocin					without oxytocin				
Pathogen	n	day 1	day 2	day 14	day 28	n	day 1	day 2	day 14	day 28
Corynebacterium bovis	9	5.95 ± 0.17 *2	5.91 ± 0.17 *0	5.68 ± 0.19 *1	5.26 ± 0.30 *0	5	5.63 ± 0.22 *1	5.55 ± 0.28 *1	5.37 ± 0.14 *0	4.97 ± 0.24 *0
Staphylococcus aureus	19	5.18 ± 0.17 *4	5.71 ± 0.10 *3	5.81 ± 0.13 *0	5.97 ± 0.13 *3	16	5.90 ± 0.16 *1	5.89 ± 0.15 *2	5.79 ± 0.16 *1	5.65 ± 0.22 *0
other Staphylococcus spp.	25	5.54 ± 0.10 *1	5.81 ± 0.09 *1	5.37 ± 0.13 *0	5.46 ± 0.15 *3	14	5.64 ± 0.17 *1	5.96 ± 0.10 *2	5.57 ± 0.13 *1	5.58 ± 0.15 *0
Streptococcus uberis	20	6.06 ± 0.17 *12	6.08 ± 0.16 *8	5.75 ± 0.20 *4	5.94 ± 0.27 *2	25	5.86 ± 0.12 *6	5.94 ± 0.10 *4	5.75 ± 0.11 *1	5.91 ± 0.13 *1
Streptococcus dysgalcatiae	6	5.73 ± 0.38 *2	5.81 ± 0.22 *1	5.62 ± 0.25 *0	5.46 ± 0.26 *0	11	6.23 ± 0.12 *6	6.28 ± 0.17 *4	5.80 ± 0.18 *1	6.15 ± 0.13 *2
Gram-negative	2	- *2	- *1	- *0	-	6	6.46 ± 0.02 *5	6.35 ± 0.08 *4	5.65 ± 0.29 *1	-

Table 3: Lactate dehydrogenase activity (U x 1000/L) in mastitis milk of udder quarters (n) in different pathogen and treatment groups (with oxytocin; without oxytocin) on different days relative to clinical diagnosis of mastitis (= day 1).

	with oxytocin						without oxytocin				
Pathogen	n	day 1	day 2	day 3	day 14	day 28	n	day 1	day 2	day 3	day 14	day 28
Corynebacterium bovis	9	0.77 ± 0.46	3.28 ± 2.07	2.01 ± 1.37	-	-	5	0.20 ± 0.09	0.23 ± 0.11	0.20 ± 0.09	-	-
Staphylococcus aureus	19	0.62 ± 0.18	0.77 ± 0.25	0.61 ± 0.17	1.48 ± 1.25	0.54 ± 0.23	16	1.13 ± 0.68	3.19 ± 2.81	0.38 ± 0.12	0.23 ± 0.09	0.43 ± 0.15
other Staphylococcus spp.	17	0.39 ± 0.14	0.73 ± 0.29	0.49 ± 0.17	0.13 ± 0.03	0.12 ± 0.04	10	0.58 ± 0.31	0.47 ± 0.22	0.55 ± 0.25	0.10 ± 0.01	0.26 ± 0.05
Streptococcus uberis	20	2.85 ± 0.75	3.30 ± 0.83	1.88 ± 0.48	0.67 ± 0.25	0.87 ± 0.30	25	1.32 ± 0.43	1.37 ± 0.40	1.16 ± 0.31	1.77 ± 0.90	-
Streptococcus dysgalcatiae	6	1.55 ± 0.15	1.71 ± 0.91	1.36 ± 0.51	0.37 ± 0.05	-	11	4.04 ± 1.75	5.48 ± 2.11	5.72 ± 2.36	1.58 ± 0.70	0.47 ± 0.13
Gram-negative	2	-	-	-	-	-	6	3.21 ± 1.55	7.79 ± 3.10	8.33 ± 2.46	4.93 ± 2.82	-

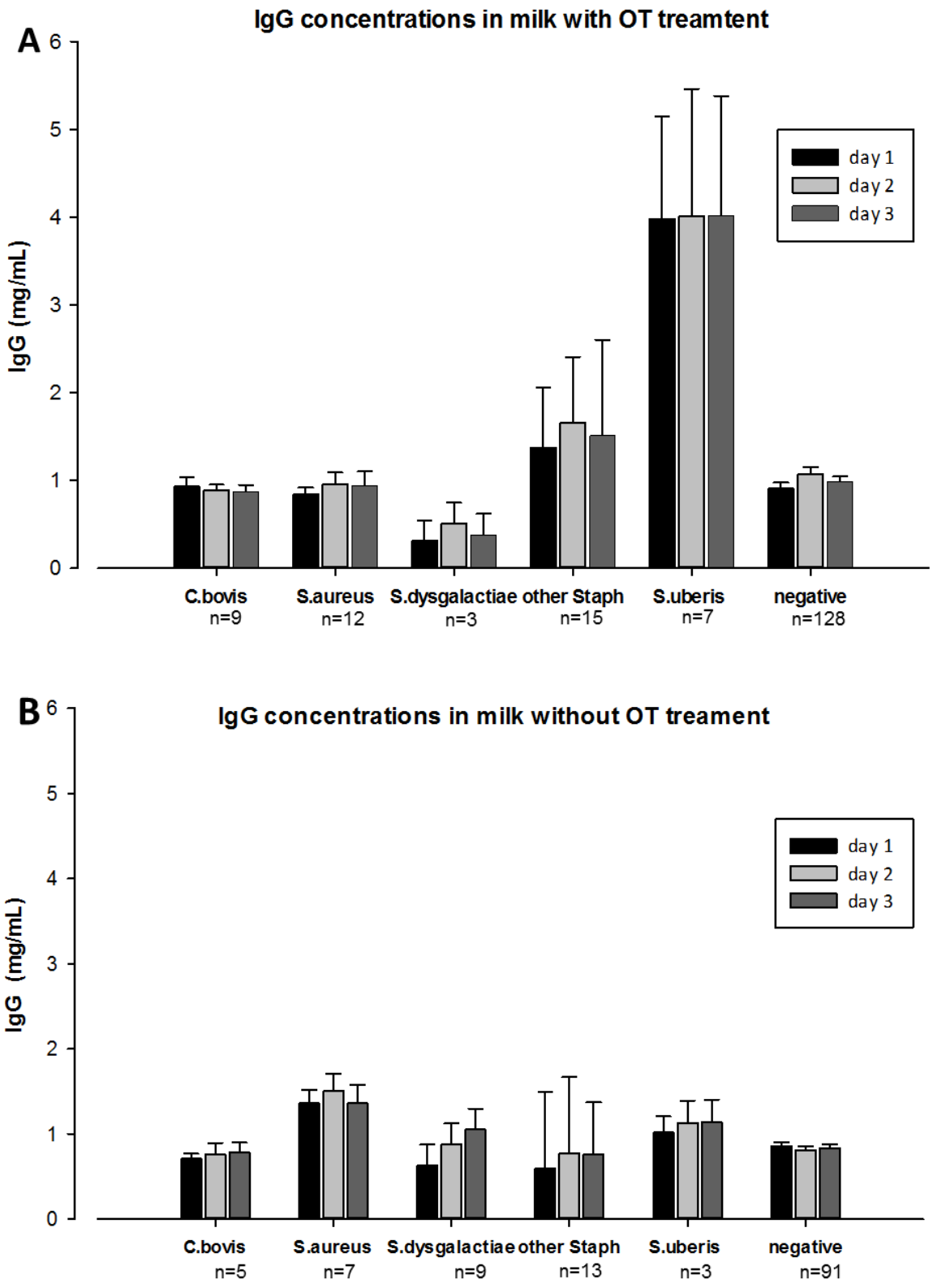

Figure 1: Immunoglobulin G concentrations in milk on days 1 (= day of clinical diagnosis of mastitis), 2, and 3 in udder quarters infected with different pathogens (C. bovis = *Corynebacterium bovis*, S. aureus = *Staphylococcus aureus*, S. dysgalactiae = *Streptococcus dysgalactiae*, other Staph = other *Staphylococcus* than *Staphylococcus aureus*, S. uberis = *Staphylococcus uberis*) with (A) or without (B) oxytocin treatment; values are means + SD; n = number of quarters.

ACKNOWLEDGEMENTS

I would like to thank all people who contributed to this work:

Prof. Dr. Heiner Bollwein, for giving me the opportunity to do this study under his supervision.

The “Tierarztpraxis Dres. Hiendl” in Zachenberg, the “VET-Praxis Oberkappel” with Hannes Pfingstner and all the dairy farmers for participating with their cows, time and support.

Prof. Dr. Dr. h.c. Roger Stephan and his team for bacteriological analyses,

Dr. Josef Gross connecting Switzerland with Bavaria and Austria, and for organizing the transport of samples to the labs in Zürich and Bern.

Dr. Maren Feldmann for advice on all open questions,

Prof. Dr. Olga Wellnitz for her great support in data analyses and manuscript preparation,

Prof. Dr. Rupert Bruckmaier who knew how to calm me down, when things seemed to go wrong, and for the analyzing of various milk constituents in his lab.

Curriculum Vitae

Vorname Name	Florian Johannes Strasser
Geburtsdatum	18.12.1989
Geburtsort	94469 Deggendorf, Deutschland
Nationalität	Deutsch
September 1996 – Juli 2000	**Grundschule Lalling**, 94551 Lalling, Deutschland
September 2000 – Juni 2009	**St.-Gotthard-Gymansium der Benediktiner Niederaltaich**, 94557 Niederalteich, Deutschland
26. Juni 2009	**Abitur**, St.-Gotthard-Gymansium der Benediktiner Niederaltaich, 94557 Niederaltaich, Deutschland
Oktober 2010 – März 2016	**Studium der Tiermedizin**, Ludwig-Maximilians-Universität München, 80539 München, Deutschland
02. März 2016	**Tierärztliche Prüfung,** Ludwig-Maximilians-Universität München, 80539 München, Deutschland
Oktober 2016 – Februar 2019	**Anfertigung der Dissertation** Unter Leitung von Prof. Dr. med. vet. Heiner Bollwein am Departement für Nutztiere der Vetsuisse-Fakultät Universität Zürich Vorsteher: Prof. Dr. med. vet. Heiner Bollwein

Fachrelevante Anstellungen nach Abschluss des tiermedizinischen Studiums

Mai 2016 – Oktober 2018	Assistenztierarzt in der tierärztlichen Praxis Dres. Hiendl, 94239 Zachenberg, Deutschland
Oktober 2018 – heute	Praxisinhaber der Straßer Tierarztpraxis für Rinder, 94551 Hunding, Deutschland